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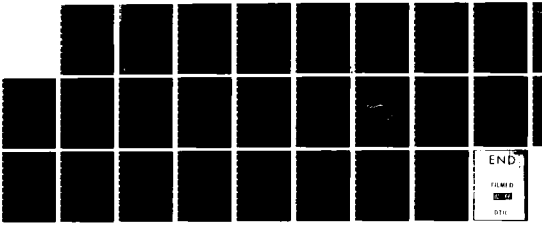

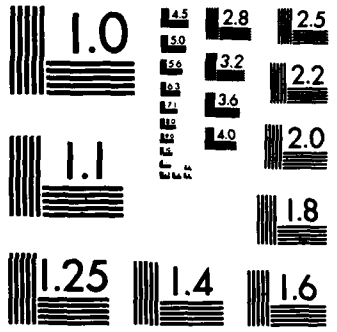

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INSTITUTE REPORT NO. 165

MUTAGENIC POTENTIAL OF 1-acetyloctahydro-3, 5, 7-trinitro-1, 3, 5, 7-tetrazocine (SEX) and 1-acetylhexahydro-3, 5-dinitro-1, 3, 5-triazine (TAX)

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LEONARD J. SAUERS, MS, SP5
and
JOHN T. FRUIN, DVM, PhD, COL VC

TOXICOLOGY GROUP,
DIVISION OF RESEARCH SUPPORT

NOVEMBER 1983

Toxicology Series 68

LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

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Mutagenic Potential of 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine (SEX) and 1-Acetylhexahydro-3,5,-Dinitro-1,3,5-Triazine (TAX) (Toxicology Series 68)--Kellner, Sauers and Fruin

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REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

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18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

Mutagenicity, Toxicology, Ames Assay, SEX, TAX, 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine, 1-Acetylhexahydro-3,5-Dinitro-1,3,5-Triazine

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

The mutagenic potential of SEX and TAX was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were exposed to doses ranging from 1 mg/plate to 10^{-5} mg/plate. Negative mutagenic responses were observed for both test compounds.

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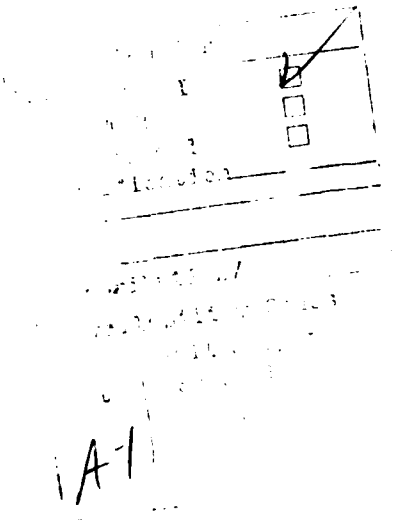
ABSTRACT

The mutagenic potential of SEX and TAX was assessed by using the Ames Salmonella/Mammalian Microsome Mutagenicity Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537, and TA 1538 were exposed to doses ranging from 1 mg/plate to 10^{-5} mg/plate. Negative mutagenic responses were observed for both test compounds.



PREFACE

TYPE REPORT: Ames Assay GLP Study Report

TESTING FACILITY: US Army Medical Research and Development Command
Letterman Army Institute of Research
Presidio of San Francisco, CA 94129

SPONSOR: US Army Medical Research and Development Command
US Army Medical Research Institute of Chemical Defense
Aberdeen Proving Ground, MD 21010

PROJECT: DMSO Recrystallization Solutions, APC TL01

GLP STUDY NUMBER: 83005

STUDY DIRECTOR: COL John T. Fruin, DVM, PhD, VC, Diplomate of
American College of Veterinary Preventive Medicine

PRINCIPAL INVESTIGATOR: SP4 Thomas P. Kellner, BA

REPORT AND DATA MANAGEMENT: A copy of the final report, study protocols, raw data, retired SOPs and an aliquot of the test compound will be retained in the LAIR Archives.

TEST SUBSTANCES: 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine (SEX)
1-Acetylhexahydro-3-5-Dinitro-1,3,5-Triazine (TAX)

INCLUSIVE STUDY DATES: 12 May - 5 June 1983

OBJECTIVE: To determine the mutagenic potential of SEX and TAX using the Ames Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were used. The plate incorporation method was followed. The test substances were diluted in reagent grade DMSO and this diluent was checked for sterility.

ACKNOWLEDGMENTS

The authors wish to thank SP4 Lawrence Mullen, BS and John Dacey for their assistance in performing the research.

Signatures of Principal Scientists involved
in the Study

We, the undersigned, believe the study number 83005 described in this report to be scientifically sound and the results and interpretation to be valid. The study was conducted to comply, to the best of our ability, with the Good Laboratory Practice Regulations outlined by the Food and Drug Administration.

Thomas Kellner / 1 Jul 83
THOMAS P. KELLNER, BA / DATE
SP4, USA
Principal Investigator

John T. Fruin July 83
JOHN T. FRUIN, DVM, PhD / DATE
COL, VC
Study Director

Leonard J. Sauers 30 June 83
LEONARD J. SAUERS, MS / DATE
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Toxicology Group

DEPARTMENT OF THE ARMY
LETTERMAN ARMY INSTITUTE OF RESEARCH
PRESIDIO OF SAN FRANCISCO, CALIFORNIA 94129

REPLY TO
ATTENTION OF:

SGRD-ULZ-QA

14 July 1983

MEMORANDUM FOR RECORD

SUBJECT: Report of GLP Compliance

I hereby certify that in relation to LAIR GLP study 83005 the following inspections were made:

19 May 83

The report and raw data for this study were audited on 13 July 1983.

Routine inspections with no adverse findings are reported quarterly, thus these inspections are also included in the July 83 report to management and the Study Director.

NELSON R. POWERS, Ph.D.
CPT, MSC
Quality Assurance Officer

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THE MUTAGENIC POTENTIAL OF: 1-Acetyloctahydro-3,5,7-Trinitro-1,3,5,7-Tetrazocine (SEX) and 1-Acetylhexahydro-3,5-Dinitro-1,3,5-Triazine (TAX)--Kellner et al

Rationale for using the Ames Assay

The Ames Salmonella/Mammalian Microsome Mutagenicity Test is one of a standard bank of tests used by our laboratory for the assessment of the mutagenic potential of a test substance. It is a short-term screening assay, which we use for the prediction of potential mutagenic agents in mammals. It is inexpensive when compared to *in vivo* tests, yet is highly predictive and reliable in its ability to detect mutagenic activity and therefore carcinogenic probability (1). It relies on basic genetic principles and allows for the incorporation of a mammalian microsomal enzyme system to increase sensitivity through enzymatically altering the test substance into an active metabolite. It has proven highly effective in assessing human risk (1).

Description of Test (Rationale for the selection of strains)

The test was developed by Bruce Ames, Ph.D. from the University of California-Berkeley. The test involves the use of several different genetically altered strains of *Salmonella typhimurium*, each with a specific mutation in the histidine operon (2). The test substance demonstrates mutagenic potential if it is able to revert the mutation in the bacterial histidine operon to the wild type and reestablish prototrophic growth within the test strain. This reversion also can occur spontaneously due to a random mutational event. If, after adding a test substance, the number of revertants is significantly greater than the spontaneous reversion rate, then the test substance physically altered the locus involved in the operon's mutation and is able to induce point mutations (2).

In order to increase the sensitivity of the test system, other mutations in the Salmonella are used (2). To insure a higher probability of uptake of test substance, the genome for the lipopolysaccharide layer (LP) is mutated and, therefore, larger molecules are allowed to enter the bacteria. Each strain has another induced mutation which causes loss of excision repair mechanisms. A mammalian microsomal enzyme system is incorporated since many chemicals are not by themselves mutagenic but have to be activated by an enzymatic process. These microsomal enzymes are obtained from

livers of rats induced with Aroclor 1254; the enzymes allow for the expression of the metabolites which would occur in the mammalian system. This activated rat liver microsomal enzyme homogenate is termed S-9.

Description of Strains (History of the strains used, method to monitor the integrity of the organisms, and data pertaining to current and historical control and spontaneous reversion rates)

The test consists of using five different strains of Salmonella typhimurium that are unable to grow in absence of histidine because of a specific mutation in the histidine operon. This histidine requirement is verified by attempting to grow the tester strains on minimal glucose agar (MGA) plates, both with and without histidine. The dependence on this amino acid is shown when growth occurs only in its presence. The plasmids in strains TA 98 and TA 100 contain an ampicillin resistant R factor. Strains deficient in this plasmid demonstrate a zone of inhibition around an ampicillin impregnated disc. The alteration of the LP layer allows uptake by the Salmonella of larger molecules. If a crystal violet impregnated disc is placed onto a plate containing any one of the bacterial strains, a zone of growth inhibition will occur because the LP layer is altered. The absence of excision repair mechanisms can be determined by using ultraviolet (UV) light. These mechanisms function primarily by repairing photodimers between pyrimidine bases. Exposure of bacteria to UV light will activate the formation of these dimers and cause cell lethality, since excision of these photodimers can not be made. The genetic mutation resulting in UV sensitivity also induces a dependence by the Salmonella to biotin. Therefore, this vitamin must be added. In order to prove that the bacteria are responsive to the mutation process, positive controls are run with known mutagens. If after exposure to the positive control substance, a revertant count is obtained which is greater than twice the spontaneous reversion rate, then the bacteria are adequately responsive. Sterility controls are performed to determine the presence of contamination. Sterility of the test compound is also confirmed in each first dilution. Verification of the tester strains occurs simultaneously with the running of each assay. The value of the spontaneous reversion rate is obtained by using the same inoculum of bacteria that is used in the assay (3).

Strains were obtained directly from Dr. Ames, University of California-Berkeley, propagated and then maintained at $-80^{o}C$ in our laboratory. Before any substance was tested, quality controls were performed on the bacterial strains to establish the presence of their special features and also to determine the spontaneous reversion rate (2). Records are maintained of all the data to determine if deviations from the set trends have occurred. These records are kept in the archives of the Quality Assurance Unit.

In this series of tests for the detection of mutagenic potential of different agents, we compared the spontaneous reversion values with our own historical values and those cited by Ames et al (2). Our conclusions are based on the spontaneous reversion rate compared to the experimentally induced rate of mutation. When operating effectively, these strains detect substances that cause base pair mutations (TA 1535, TA 100) and frameshift mutations (TA 1537, TA 1538, and TA 98).

Objective of Study

The objective of the study is to determine the mutagenic potential of SEX and TAX by using the Ames Assay. Tester strains TA 98, TA 100, TA 1535, TA 1537 and TA 1538 were used. The plate incorporation method was followed. The test substances were diluted in reagent grade DMSO and this diluent was checked for sterility.

METHODS (3)

Rationale for Dosage Levels and Dose Response Tabulations

To insure readable and reliable results, a sublethal concentration of the test substance had to be determined. This toxicity level was found by using MGA plates, various concentrations of the substance, and approximately 10^8 cells of TA 100 per plate, unless otherwise specified. Top agar containing trace amounts of histidine and biotin were placed on MGA plates. TA 100 was used because it is the most sensitive strain. Strain verification was confirmed on the bacteria, along with a determination of the spontaneous reversion rate. After incubation, the growth was observed on the plates. (The auxotrophic Salmonella will replicate a few times and potentially express a mutation. When the histidine and biotin supplies are exhausted, only those bacteria that reverted to the prototrophic phenotype will continue to reproduce and form macrocolonies; the remainder of the bacteria comprises the background lawn. The minimum toxic level is defined as the lowest serial dilution at which decreased macrocolony formation, below that of the spontaneous revertant rate, and an observable reduction in the density of the background lawn occurs.) A maximum dose of 1 mg/plate is used when no toxicity is observed. The densities were recorded as normal, slight, and no growth.

Test Format

After we validated our bacterial strains and determined the optimal dosage of the test substance, we began the Ames Assay. In the actual experiment, 0.1 ml of the particular strain of Salmonella (10^8 cells) and the specific dilutions of the test substance were added to 2 ml of molten top agar, which contained trace amounts of histidine and biotin. Since survival is better from cultures which have just passed the log phase, the Salmonella strains are used 16 hours

(maximum) after initial inoculation into nutrient broth. The dose of the test substance spanned 10^5-fold, decreasing from the minimum toxic level by a dilution factor of 10. All the substances were tested with and without S-9 microsome fraction. The optimal titer of the S-9 was determined and 0.5 ml was added to the molten top agar. After all the ingredients were added, the top agar was mixed, then overlaid on minimum glucose agar plates. These plates contained 2% glucose and Vogel Bonner "E" Concentrate (4). The water used in this medium and all reagents came from a polymetric system. Plates were incubated upside down in the dark at 37° C for 48 hours. Plates were prepared in triplicate and the average revertant counts were recorded. The corresponding number of revertants obtained was compared to the number of spontaneous revertants; the conclusions were recorded statistically. A correlated dose response is considered necessary to declare a substance as a mutagen. Commoner (5), in his report, "Reliablilty of Bacterial Mutagenesis Techniques to Distinguish Carcinogenic and Non-Carcinogenic Chemical," and McCann et al (1) in their paper, "Detection of Carcinogens as Mutagen in the Salmonella/Mammalian Microsome Mutagenicity Test: Assay of over 300 Chemicals," have concurred on the test's ability to detect mutagenic potential.

Statistical Analysis

Quantitative evaluation was ascertained by the method of Ames (2). He assumed that a compound which causes twice the spontaneous reversion rate and a correlated dose response is mutagenic.

Chemical Analysis

Information on the chemical analysis of SEX and TAX was obtained from the source of the chemicals, SRI International (Appendix A).

RESULTS AND DISCUSSION

Throughout this report 1-acetyloctahydro-3,5,7-trinitro-1,3,5,7-tetrazocine will be referred to as SEX and 1-acetylhexahydro-3,5-dinitro-1,3,5-triazine will be called TAX.

On 12 May 1983 the toxicity level determination was performed on all the test compounds. All sterility, strain verification, and negative controls were normal (Table 1). No toxicity was observed for either test chemical at the highest dose of 1 mg/plate (Tables 2 and 3).

On 21 May 1983, the Ames Assay was performed on SEX and TAX. All strain verification and sterility controls were normal for this experiment (Table 4). Expected results were obtained for all positive and negative controls (Table 5). The bacterial strains were exposed to doses ranging from 1 mg/plate to 10^{-5} mg/plate of test substance.

In the case of SEX, a doubling of the spontaneous reversion rate did occur at the highest dose level (1 mg/plate) for strains TA 1535 and TA 1537 with S-9 added (Table 6). However, TA 100, a more sensitive indicator of basepair mutagens than TA 1535, did not show a doubling of the spontaneous reversion rate. TA 98 and TA 1528, detectors of frameshift mutagens as TA 1537, showed no doubling of the spontaneous reversion rate. Also, no dose response was elicited by SEX on the strains tested. Based on these data, SEX is not mutagenic at the dose levels tested. In no case was a dose response or a doubling of the spontaneous reversion rate observed for TAX (Table 7). Tables 1-7 appear in Appendix B.

CONCLUSION

Based on the Ames Assay, SEX and TAX are not mutagenic at the levels tested.

RECOMMENDATION

None.

REFERENCES

1. McCann JE, Choi E, Yamasaki E, Ames BN. Detection of carcinogens as mutagens in the Salmonella/microsome test: Assay of 300 chemicals. Proc Nat Acad Sci, USA 1975;72:5135-5139.

2. Ames BN, McCann J, Yamasaki E. Methods for detection of carcinogens and mutagens with Salmonella/mammalian microsome mutagenicity test. Mutat Res 1975;31:347-364.

3. LAIR SOP OP-STX-1, Ames Salmonella/mammalian microsome mutagenicity test.

4. Vogel HJ, Bonner DM. Acetylornithinase of E. coli: Partial purification and same properties. J Biol Chem 1956;218:97-106.

5. Commoner B. Reliability of the bacterial mutagenesis techniques to distinguish carcinogenic and non-carcinogenic chemicals. EPA 600/1 76-002, 1976.

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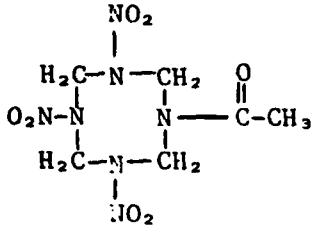
APPENDICES

FROM: Preparation and Purification of Multigram Quanitites of TAX and SEX, Bedford et al, Organic Chemistry Department, SRI International, 333 Ravenwood Avenue, Menlo Park, CA 94025

CHARACTERIZATION OF SEX

SEX appears sufficiently stable in normal nitrolysis media to exist as a contaminant in RDX/HMX manufacturing process. The characteristics of SEX are as follows:

Structural Formula:



Empirical Formula: $C_6H_{11}N_7O_7$

Elemental Analysis: Calculated: C, 24.57; H, 3.75; N, 33.45
C, 24.21; H, 3.76; N, 33.45

Melting Point: 237°-237.5°C (DEC)

Density: 1.785 g/cm³ at 21°C

Molecular Weight: 293 (Calculated)

Solubility: Soluble in dimethylsulfoxide. Slightly soluble in acetone, nitromethane, and acetonitrile. Almost insoluble in ethanol, benzene, and ether.

Impact Sensitivity (drop weight test): Greater than 300 kg-cm compared with 148 kg-cm for pure HMX. SEX is sensitive to direct strong hammer blows. During our investigations SEX has exhibited no instability, but because of the hammer results should be handled as a potential explosive, like HMX.

Infrared Spectrum: See Figure 7.

Proton NMR Spectrum: See Figure 8.

Chemical Properties: SEX gives a positive Franchimont nitramine reaction, but a negative Liebermann nitroso test. Decomposition in hydroxide fails to produce free CH_3COO^- for a lanthanum nitrate test.

However, if SEX is decomposed in 96% sulfuric acid, the distillate gives a lanthanum nitrate test.[7]

SEX appears inert to boiling acetic anhydride and unaffected by treatment with ammonium nitrate-nitric acid mixtures. Absolute nitric acid at 50°-60°C converts SEX to HMX. Warm 70% nitric acid destroys the compound rapidly, as does 10% aqueous sodium hydroxide and 28% ammonia.

Purity: The purity of SEX was determined by analytical HPLC with a Spectra-Physics 3500B Liquid Chromatograph. A waters RCM-100, C_{18} cartridge with a mobile phase of 80/20 water/methanol was used for DADN/SEX/HMX mixtures. An internal standard of RDX was used with $1/R_f$* values of 1.5 for HMX, 1.5 for SEX, and 1.7 for DADN. Hot-column chromatographed SEX contained no detectable amounts of DADN (starting material) and only 1% to 2% HMX (sole contaminant). High pressure liquid chromatographed material contained no DADN or HMX. Also, no other contaminants were detected by analytical HPLC, ensuring a 99.9+% purity of SEX.

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*R_f = response factor.

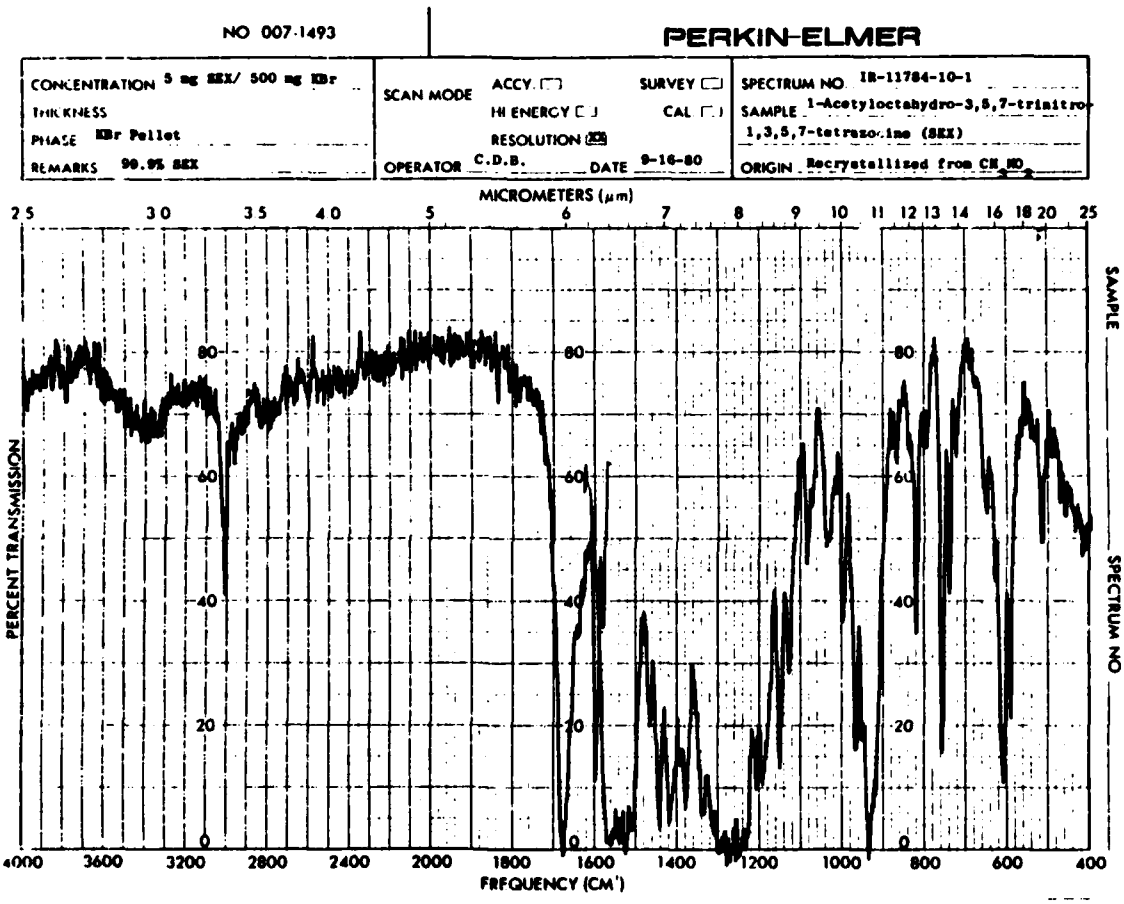


FIGURE 7 INFRARED SPECTRUM OF 99.9+% HEX

APPENDIX A (cont.)

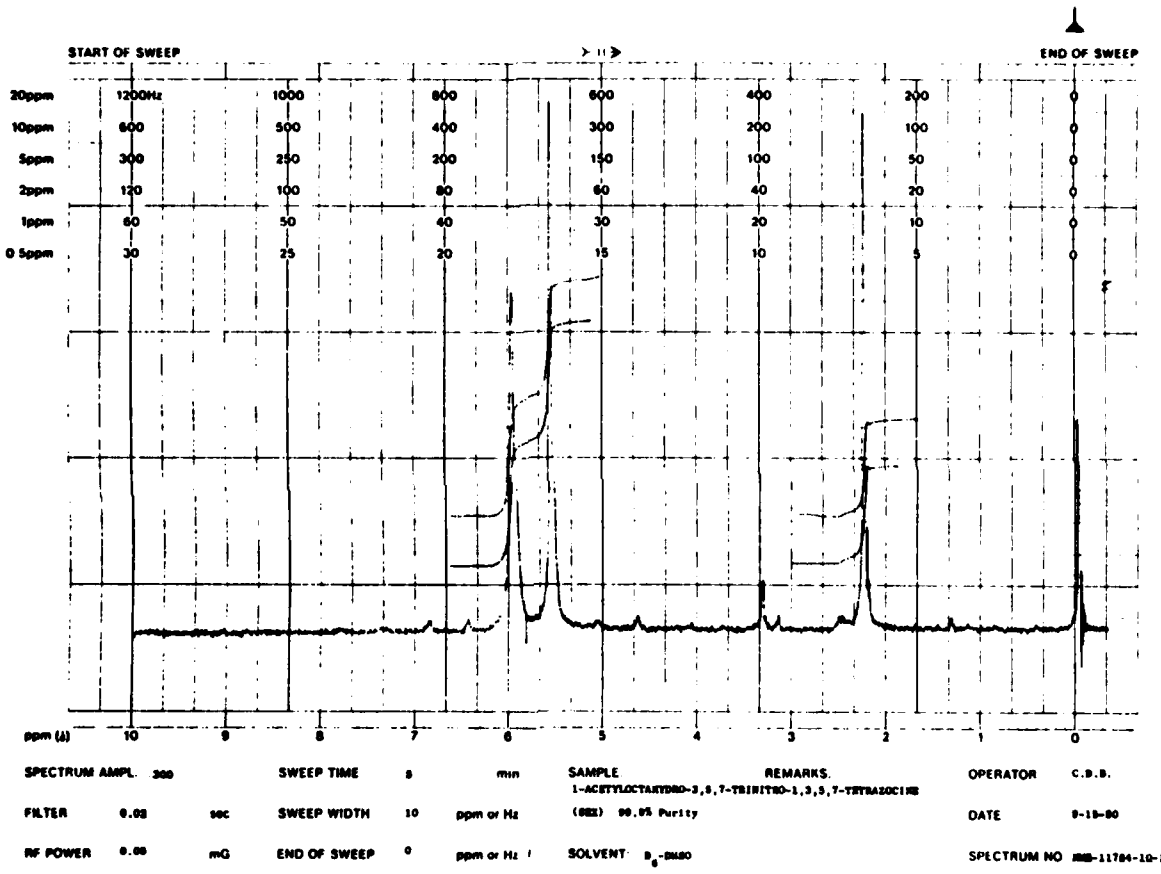


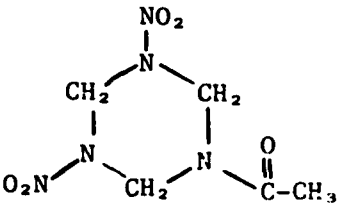
FIGURE 8 PROTON NMR SPECTRUM OF 99.9+% HMX

APPENDIX A (cont.)

CHARACTERIZATION OF TAX

TAX appears sufficiently stable in normal nitrolysis media to exist as a contaminant in RDX/HMX manufacturing process. The characteristics of TAX are as follows:

Structural Formula:



Empirical Formula: $C_5H_9N_5O_5$

Elemental Analysis: Calculated: C, 27.39; H, 4.11; N, 31.96
Found: C, 27.45, 27.40; H, 4.14, 4.16; N, 31.75, 31.87

Melting Point: 158°-159°C

Density: 1.675 g/cm^3 at 21°C

Molecular Weight: 219 (Calculated)

Solubility: Soluble in acetone, acetonitrile, methanol, ethanol, and nitromethane. Insoluble in trifluoroacetic acid.

Impact Sensitivity (drop weight test): Greater than 300 kg-cm compared with 134 kg-cm for pure RDX. TAX is insensitive to direct strong hammer blows. During our investigations TAX has not exhibited any impact sensitivity.

Infrared spectrum: See Figure 5.

Proton NMR Spectrum: See Figure 6.

Chemical Properties: TAX is destroyed rapidly by 96% sulfuric acid.

Purity: The purity of TAX was determined by analytical HPLC using a reverse-phase system with 30/70 methanol/water eluent. An internal standard of 1,3,5-trinitrobenzene was used with $1/R_f$ values of 2.39 for RDX and 3.21 for TAX. Column chromatographed TAX contained no detectable amounts of TRAT (starting material) or RDX (major contaminant of crude reaction mixtures). Also, no other contaminants were detected by HPLC, ensuring a 99.9+% purity of material.

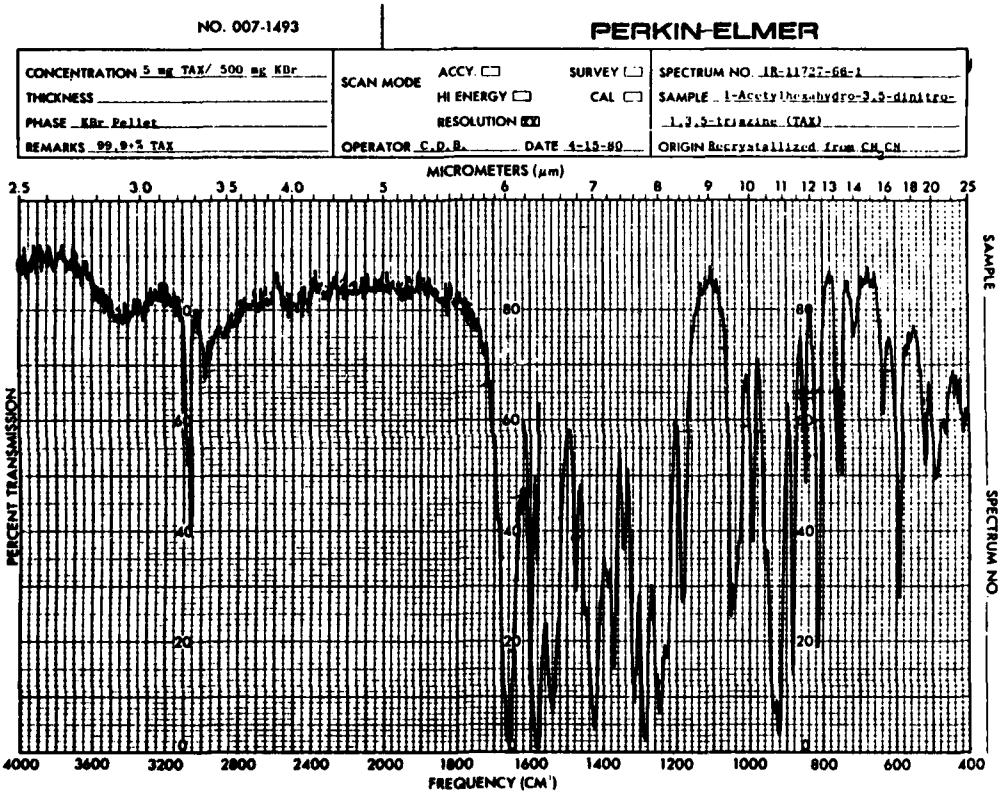


FIGURE 5 INFRARED SPECTRUM OF 99.9% TAX

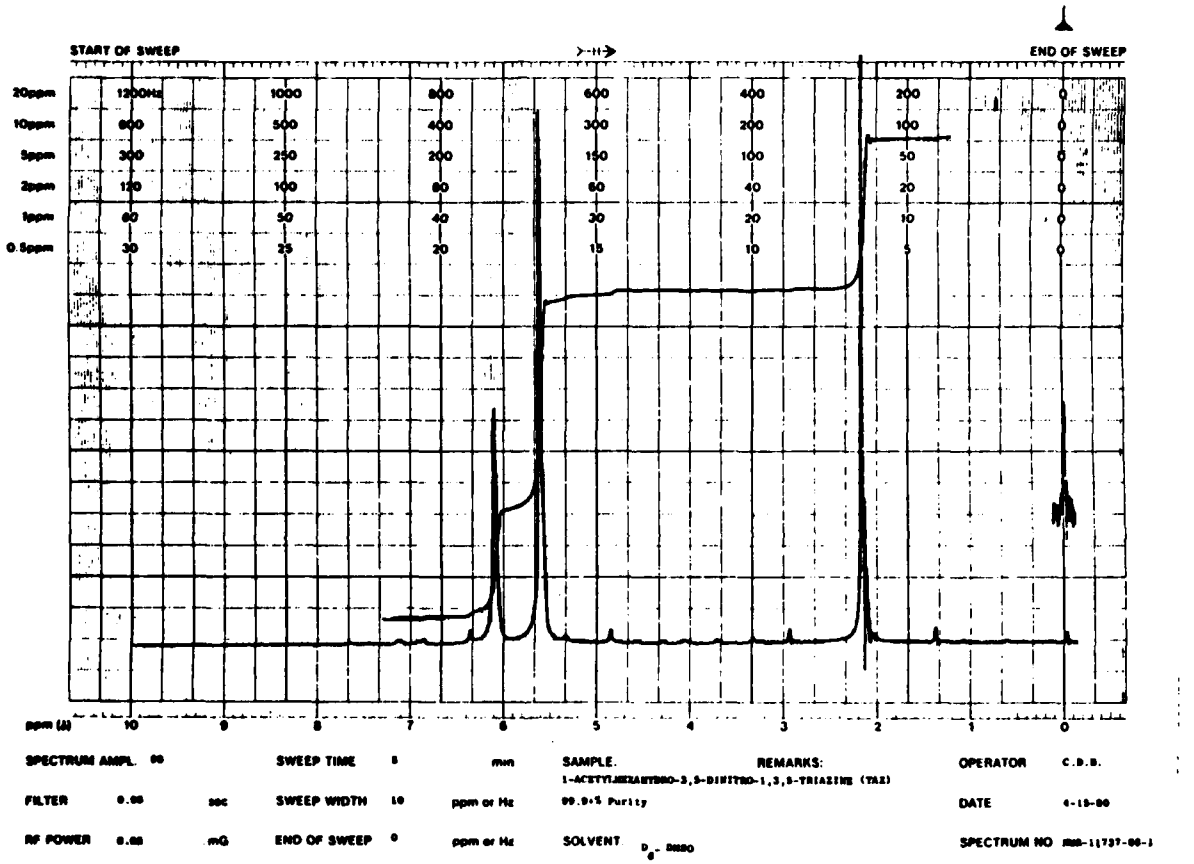


FIGURE 6 NMR SPECTRUM OF 99.9% TAX

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Table 7	Number of Revertants/Plate (TAX)	21 May 83	26

TABLE 1

STRAIN VERIFICATION FOR TOXICITY LEVEL DETERMINATION

Strains	Histidine Requirement	Ampicillin Resistance	UV Sensitivity to Crystal Violet		Sterility Control	Response (1)
100	NG	G	NG	16 mm	NG	+
1537	NG	16.5 mm	NG	15 mm	NG	+
WT	G	NG	G	GG	NA	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG MGA Plate: NG

Top Agar Initial: NG End: NG

Diluent: NG Nutrient Broth: NG

Test Compound (a) SEX (b) TAX (c) N/A (d) N/A (e) N/A

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

Spontaneous Revertants: TA 100, No S-9 90, 110, 101

(1) + = expected response - = unexpected response

Study Number: 83005 Date: 13 MAY83 By: KELLNER

TABLE 2

TOXICITY LEVEL DETERMINATION

Substance assayed: SEX Substance dissolved in: DMSO

Study Number: 83005 Date: 14 MAY 83 Performed by: KELLNER

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
1 mg/plate	96	94	104	98	NL
0.1 mg/plate	109	109	86	101	NL
10^{-2} mg/plate	91	96	113	100	NL
10^{-3} mg/plate	77	110	87	91	NL
10^{-4} mg/plate	90	83	75	83	NL
10^{-5} mg/plate	92	90	94	92	NL
10^{-6} mg/plate	67	117	100	98	NL
10^{-7} mg/plate	85	116	79	93	NL

(1) NG = No Growth ST = Slight Growth NL = Normal Lawn

TABLE 3

TOXICITY LEVEL DETERMINATION

Substance assayed: TAX Substance dissolved in: DMSO

Study Number: 83005 Date: 14 MAY 83 Performed by: KELLNER

TA 100 REVERTANT PLATE COUNT

Test Compound Concentration	Plate #1	Plate #2	Plate #3	Average	Background Lawn (1)
1 mg/plate	94	90	73	86	NL
0.1 mg/plate	88	109	87	95	NL
10^{-2} mg/plate	78	81	91	83	NL
10^{-3} mg/plate	75	89	83	82	NL
10^{-4} mg/plate	112	85	CON	98	NL
10^{-5} mg/plate	81	93	104	93	NL
10^{-6} mg/plate	88	83	101	91	NL
10^{-7} mg/plate	110	94	81	95	NL

(1) NG = No Growth ST = Slight Growth NL = Normal Lawn

APPENDIX B (cont.)

TABLE 4

STRAIN VERIFICATION CONTROL

Strains	Histidine Requirement	Ampicillin Resistance	Sensitivity to UV	Sensitivity to Crystal Violet	Sterility Control	Response (1)
98	NG	G	NG	16 mm	NG	+
100	NG	G	NG	18 mm	NG	+
1535	NG	30 mm	NG	17 mm	NG	+
1537	NG	25 mm	NG	19 mm	NG	+
1538	NG	30 mm	NG	20 mm	NG	+
WT	G	NA	G	G	NA	+

STERILITY CONTROL

His-Bio Mix Initial: NG End: NG Diluent: NG

Top Agar Initial: NG End: NG MGA Plate: NG

S-9 Mix Initial: NG End: NG Nutrient Broth: NG

Test Compound (a) SEX (b) TAX (c) N/A (d) N/A (e) N/A (f) N/A

G = Growth NG = No Growth NT = Not Tested NA = Not Applicable WT = Wild Type

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(1) + = expected response
- = unexpected response

TABLE 5

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
AF	2 ug/plate	yes	(511,580,438) 510	(487,589,291) 456			(462,711,610) 594
BP	2 ug/plate	yes	(799,585,401) 595	(856,751,714) 774		(76,107, 89) 91	(136,117,149) 134
AA	2 ug/plate	yes	(999,999,999)* 999	(999,999,999)* 999		(262,247,252) 254	(999,999,999)* 999
MNNG	2 ug/plate	no		(999,999,999)* 999			
	20 ug/plate	no			(999,999,999)* 999		
Spontaneous Reversion Rate							
	before	yes	(17, 25, 20)	(134,110,124)	(9, 11, 10)	(3, 7, 5)	(13, 16, 17)
	after		(22, 26, 15) 21	(106, 88,107) 112	(11, 8, 7) 9	(8, 2, 2) 4	(19, 11, 20) 16
	before	no	(14, 15, 13)	(104,121,107)	(14, 13, 13)	(8, 7, 4)	(12, 14, 9)
	after		(18, 18, 9) 14	(101, 89, 87) 102	(14, 10, 15) 13	(3, 4, 4) 5	(15, 17, 20) 14

* A value of 999 represents a count of over 1000

Study Number: 83005

Date: 21 May 83 By: Kellner, Sauers, Dacey, Mullen

TABLE 6

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
SEX	1 mg/plate	yes	(22, 25, 24) 24	(127,119,129) 125	(17, 21, 20) 19	(10, 10, 17) 12	(20, 18, 23) 20
		no	(15, 17, 20) 17	(111,100,104) 105	(19, 17, 37) 24	(6, 10, 5) 7	(17, 11, 17) 15
	0.1 mg/plate	yes	(24, 21, 21) 22	(118,126,116) 120	(13, 6, 18) 12	(4, 4, 5) 4	(18, 20, 15) 18
		no	(22, 8, 29) 20	(100,116,115) 110	(19, 9, 20) 16	(7, 5, 5) 6	(19, 15, 18) 17
	10^{-2} mg/plate	yes	(38, 18, 21) 26	(99,115,123) 112	(19, 16, 8) 14	(5, 4, 7) 5	(19, 20, 20) 20
		no	(20, 19, 23) 21	(105,101, 94) 100	(17, 29, 15) 20	(10, 8, 9) 9	(20, 17, 22) 20
	10^{-3} mg/plate	yes	(24, 36, 25) 28	(106, 99,130) 112	(9, 7, 17) 11	(5, 4, 6) 5	(27,c. , 30) 28
		no	(18, 19, 24) 20	(108, 94,103) 102	(20, 25, 14) 20	(2, 2, 4) 3	(12, 8, 17) 12

c. = Plate count not obtained due to contamination

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APPENDIX B (cont.)

TABLE 6 (concluded)

NUMBER OF REVERTANTS/PLATE

Compd	Amount of Compd. Added	S-9 Added	Strain No. 98	100	1535	1537	1538
SEX	10^{-4} mg/plate	yes	(25, 26, 25) 25	(94, 93, 99) 95	(13, 15, 11) 13	(10, 10, 7) 9	(19, 20, 23) 21
		no	(21, c., 25) 23	(105, c., 91) 98	(26, 23, 24) 24	(7, 6, 8) 7	(15, 21, 20) 19
	10^{-5} mg/plate	yes	(32, 29, 27) 29	(113, 82, 101) 99	(7, 11, 7) 8	(7, 6, 7) 7	(17, 15, 19) 17
		no	(18, 11, c.) 14	(102, 99, 114) 105	(20, 19, 28) 22	(5, 3, 4) 4	(11, 17, c.) 14

c. = Plate count not obtained due to contamination

Study No.: 83005 Date: 21 May 83 Performed by: Kellner, Sauers, Dacey, Mullen

TABLE 7

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
TAX	1 mg/plate	yes	(23, 29, 14) 22	(107, 100, 94) 100	(15, 13, 8) 12	(7, 5, 6) 6	(16, 17, 17) 17
		no	(11, 13, 8) 11	(81, 75, 60) 72	(8, 9, 13) 10	(4, 2, 5) 4	(10, 17, 7) 11
	0.1 mg/plate	yes	(15, 17, 12) 15	(110, 96, 91) 99	(4, 11, 11) 9	(3, 8, 3) 5	(12, 10, 10) 11
		no	(14, 9, 21) 15	(80, 86, 74) 80	(12, 13, 8) 11	(3, 3, 4) 3	(12, 11, 12) 12
	10^{-2} mg/plate	yes	(15, c., 12) 14	(103, 101, 72) 92	(8, 5, 15) 9	(7, c., 2) 4	(22, 20, 19) 20
		no	(9, 10, 11) 10	(103, 85, 101) 96	(13, 9, 8) 10	(6, 6, 5) 6	(6, 14, 8) 9
	10^{-3} mg/plate	yes	(20, 22, 15) 19	(81, 118, 101) 100	(9, 7, 9) 8	(7, 7, 5) 6	(21, 20, 24) 22
		no	(9, 13, 10) 11	(84, 79, 81) 81	(10, 15, 16) 14	(5, 1, 4) 3	(10, 10, 12) 11

c. = Plate count not obtained due to contamination

Study Number: 83005 Date: 21 May 83 By: Kellner, Sauers, Dacey, Mullen

TABLE 7 (concluded)

NUMBER OF REVERTANTS/PLATE

Compd.	Amount of Compd. Added	S-9 Added	Strain Number 98	100	1535	1537	1538
TAX	10^{-4} mg/plate	yes	(19, 23, 18) 20	(101, 114, 99) 105	(13, 11, 10) 11	(8, 5, 6) 6	(24, 14, 27) 22
		no	(11, 10, 11) 11	(100, 90, 91) 94	(10, 12, 11) 11	(5, 5, 2) 4	(13, 13, 19) 15
	10^{-5} mg/plate	yes	(10, 14, 17) 14	(103, 104, 105) 104	(9, 11, 7) 9	(6, 9, 3) 6	(17, 13, 11) 14
		no	(9, 3, 14) 9	(96, 97, 80) 91	(10, 9, 10) 10	(5, 4, 7) 5	(17, 9, 9) 12

Study Number: 83005 Date: 21 May 83 By: Kellner, Sauers, Dacey, Mullen

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